NOTES

Biocompatible Block Copolymers Composed of Polydimethylsiloxane and Poly[(2-methacryloyloxy)ethyl phosphorylcholine] Segments

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Some graft and block copolymers containing the polydimethylsiloxane (PDMS) segment have been synthesized so far, for the improvement of mechanical property and the biocompatibility of silicone rubber as a useful biomedical material.^{1–4} On the other hand, there is no doubt that incorporation of the polar head group of phosphorylcholine into a polymer is useful for providing materials with biocompatibility. In addition to the homopolymer (PMPC)⁵ of 2-(methacryloyloxy)-ethyl phosphorylcholine (MPC), copolymers consisting of MPC and alkyl methacrylates, for instance, show reduced protein adsorption from human plasma and reduced activation of platelets.^{6–10} On this note a novel PDMS type of block copolymers (PMPC-*b*-PDMS) composed of PMPC and PDMS segments was prepared by radical polymerization of MPC initiated with a PDMS type of azo-initiator.^{11,12} The biocompatibility of PMPC-*b*-PDMS was evaluated by the adsorption of protein and the adhesion of human platelets.

EXPERIMENTAL

Reagents

The macroazo-initiator (VPS: $M_n = 3 \times 10^4$ – 4×10^4) was kindly supplied by Wako Pure Chemicals and used without further purification. MPC was synthesized as described in a previous paper.⁹ Crystallized and lyophilized bovine serum albumin (Alb, Sigma Chemicals), human serum γ -globulin (Glo, Wako Pure Chemicals) and bovine serum fibrinogen (Fib, Sigma Chemicals) were used as obtained.

Polymerization

A series of PMPC-*b*-PDMS-I–VI with different molar ratios of PMPC segment to PDMS segment ($p:q$) was prepared by radical polymerization of MPC initiated with VPS in a mixed solvent of CHCl_3 and ethanol (70:30 vol%) at 60°C for 20 h, varying the molar ratio of MPC to the dimethylsiloxane (DMS) unit in VPS from 0.05:0.95 to 0.50:0.50 (Scheme 1 and Table I). PMPC-*b*-PDMS was purified by reprecipitation from the ethanol–diethylether system twice.

Protein Adsorption Procedure

The amount of adsorbed protein was measured by the same method described in a previous paper.¹³ The column (20 mm ϕ , 300 mm in height) used was filled with 83.34 g (1.0 m²) of glass beads surface-covered with PMPC-*b*-PDMS, which were prepared by immersing the glass beads (0.20 mm ϕ , 1.0 m² of total surface area) in the 1 wt% ethanol solution of PMPC-*b*-PDMS and drying them in a vacuum at room temperature for 24 h. The amount of protein adsorbed on the glass beads was calculated from the content of the free protein remaining in the phosphate buffer solution (PBS, pH 5.6 for Alb; pH 6.2 for Glo; pH 5.7 for Fib) by measuring the absorbance at 750 nm of protein, according to the Lowry method.¹⁴

Platelet Adhesion

Platelet-rich plasma (PRP) and platelet-poor plasma (PPP) were collected from healthy humans according to the method described in literature.¹⁰ The PRP was diluted with PPP to control the number of platelets (12.7×10^7 cells cm⁻³). The poly(ethylene terephthalate) (PET) film coated with PMPC-*b*-PDMS-V (see Table I) was placed on the bottom of the well (Corning 24 mutiwells, diameter: 15 mm) and put in contact with 0.6 mL of PRP at room temperature for 3 h. The PET film was rinsed with 1 mL of phosphate-buffered solution (PBS, pH 7.4) twice to remove the unadhered platelets, and then immersed in PBS containing the 2 wt% of glutaraldehyde to fix the adhered platelets. The film was then freeze-dried and coated with gold using a JEOL JFC-1100E ion sputter apparatus.

Other Measurements

¹H NMR spectra were recorded on a JEOL EX 400 spectrometer. Scanning electron microscope (SEM) and transmission electron microscope (TEM) images were taken by means of JEOL JSM-82 and JEOL 2000 FX, respectively. The surface of the vacuum-dried and freeze-dried films, which were prepared by casting the 3% ethanol solution of PMPC-*b*-PDMS on the PET film, was analyzed by means of X-ray photoelectron spectroscopy (XPS, Shimadzu ESCA 750) using Mg- K_α in a vacuum of less than 6.6×10^{-5} Pa. The static contact

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Scheme 1. Preparation of PMPC-*b*-PDMS.Table I. Preparation and characterization of PMPC-*b*-PDMS^a

PDMS- <i>b</i> -PMPC	MPC : DMS (Molar ratio in feed)	Yield %	$[\eta]^b$ dL g ⁻¹	$p : q^c$ (Molar ratio in block copolymer)	Property
I	0.05 : 0.95	90.3	0.12 ^d	0.03 : 0.97 ^e	Transparent rubbery
II	0.08 : 0.92	89.7	0.13 ^d	0.07 : 0.93 ^e	White rubbery
III	0.17 : 0.83	90.6	0.15	0.15 : 0.85	White rubbery
IV	0.23 : 0.76	90.4	0.15	0.20 : 0.80	White powdery
V	0.27 : 0.73	88.0	0.17	0.25 : 0.75	White powdery
VI	0.50 : 0.50	81.3	0.22	0.48 : 0.52	White powdery

^a Polymerized with VPS containing 8.47 mmol of DMS at 60°C on 10 mL of CHCl₃ : C₂H₅OH (70 : 30 vol%) for 20 h. ^b Measured in CH₃OH at 25°C. ^c Calculated from ¹H NMR in CD₃OD. ^d Measured in CHCl₃. ^e Measured in CDCl₃.

angles (θ) of water were measured at an ambient temperature using the sessile drop method with an Erma contact angle meter, Model G-1, using the PET film covered with PMPC-*b*-PDMS as described above. UV-Vis measurements were performed with a Shimadzu UV-160A spectrophotometer.

RESULTS AND DISCUSSION

Synthesis and Characterization of PMPC-*b*-PDMS

A series of block copolymers, PMPC-*b*-PDMS-I—VI, was obtained at a high yield, as shown in Scheme 1 and Table I. The molar ratio of the PMPC segment to the PDMS segment ($p : q$) in block copolymers was determined from the comparison of the peak area for COOCH₂CH₂ (3.80 ppm) of PMPC with that for (CH₃)₂SiO (0.15 ppm) of PDMS by ¹H NMR spectroscopy. The $p : q$ in PMPC-*b*-PDMS agrees closely with the molar ratio of MPC to DMS in feed. The series of PMPC-*b*-PDMS varied in appearance from rubbery to white powdery, depending on the amount of the PMPC segment (p -value). The solubility of PMPC-*b*-PDMS was also changed by the p -value as follows: PMPC-*b*-PDMS with $p \leq 0.07$ was soluble in various organic solvents such as CHCl₃ and benzene, whereas PMPC-*b*-PDMS with $p \geq 0.15$ was soluble in ethanol and insoluble but swelled up in water. The surface of the freeze-dried and vacuum-dried PMPC-*b*-PDMS-V film (see Table I) was analyzed by XPS. The signals for silicon (Si_{2p}) at a binding energy (EB) of 102 eV, phosphorus (P_{2p}) at an EB of 133 eV, and nitrogen (N_{1s}) at an EB of 400 eV

Table II. XPS analysis data for PMPC-*b*-PDMS-V

PMPC- <i>b</i> -PDMS-V	N/C × 10 ³	P/C × 10 ³	Si/C × 10 ³
XPS vacuum-dried	13	3	327
XPS freeze-dried	35	23	273

were assigned to the DMS unit in the PDMS segment and the phosphorylcholine group in the MPC moiety. It was found from the comparison of the freeze-dried surfaces with the vacuum-dried ones that the atom ratios for nitrogen and phosphorus to carbon were found to increase from N/C = 13 × 10⁻³ to N/C = 35 × 10⁻³ and from P/C = 3 × 10⁻³ to P/C = 23 × 10⁻³, respectively, as shown in Table II. Taking into account the fact that the hydrophilic segment tends to reorganize to minimize the energy depending on the surface condition in the copolymer consists of two kinds of segments with different surface free energy,^{2,15} a preferential existence would be expected for the PMPC segment of PMPC-*b*-PDMS at the water-contacting interface.⁹ The silicone rubber sheds water well, while PMPC-*b*-PDMS shows an improved wettability due to the PMPC segment. The static contact angles (θ) were measured in order to evaluate the wettability of PMPC-*b*-PDMS. Figure 1 shows the effect of the amount of PMPC segment (p) on the contact angle of the PMPC-*b*-PDMS. The static contact angles of PMPC-*b*-PDMS dropped from $\theta = 103^\circ$ to $\theta = 25^\circ$ when increasing the amount of the MPC segment. This means that PMPC-*b*-PDMS can form a hydrogel structure in water and exhibit the biocompat-

ibility mentioned below. It is also known that the kind of block copolymers such as poly(2-hydroxyethyl methacrylate)-polystyrene-poly(2-hydroxyethyl methacrylate) triblock copolymer¹⁶ and segmented polyetherurethaneurea¹⁷ have a microphase separated structure and exhibit an excellent antithrombogenic property. PMPC-*b*-PDMS-V (see Table I) was then morphologically analyzed with a TEM in order to confirm such a structure after staining the sliced surface of the sample with RuO₄ vapor. PMPC-*b*-PDMS-V was found to have a microphase separated structure¹⁵ with the domain size ranging from several ten to several hundred nanometers, as shown in Figure 2.

Biocompatibility of PMPC-*b*-PDMS

It is generally accepted that the plasma protein adsorption, which is the initial event in blood-material interaction, will influence the subsequent platelet adhesion and activation. Therefore, the interaction between PMPC-*b*-PDMS and the serum proteins such as Alb, Glo, and Fib was studied to obtain basic information for biomedical applications, by using the glass beads method¹³: at pH 5.6, pH 6.2, and pH 5.6 for Alb, Glo, and Fib, respectively. These pH values are close to the respective isoelectric points of the proteins.

Figure 1. Effect of the molar ratio of MPC segment (p) in PMPC-*b*-PDMS on the contact angle of water.

The amount of adsorption of proteins decreased with increasing p -value, as shown in Figure 3. It is noteworthy that PMPC-*b*-PDMS effectively suppresses the adsorption of Fib, which is recognized as an important blood coagulation factor.¹⁵ Blood compatibility was

Figure 2. TEM micrograph of PMPC-*b*-PDMS-V.

Figure 3. Adsorption of protein on PMPC-*b*-PDMS; [Protein]₀ = 50 mgL⁻¹, pH 5.6 for Alb, pH 6.2 for Glo, pH 6.0 for Fib, ionic strength 0.01, 2 h. ▲, Alb; ●, Glo; ■, Fib.

PDMS-*b*-PMPC-V

silicone

Figure 4. SEM micrographs of PDMS-*b*-PMPC-V and silicone after contact with PRP for 180 min.

also evaluated by the adhesion of human platelets. Figure 4 exhibits the SEM micrograph of PMPC-*b*-PDMS-V after contact with PRP for 180 min, together with the case of silicone rubber as a control. No activation of platelets occurs on the PMPC-*b*-PDMS-V, whereas platelets aggregate and deform on silicone rubber. In conclusion, PDMS-*b*-PMPC is expected to be a new material having both a hydrogel structure and a micro-phase-separation structure for biomedical uses.

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